JX32

VOLUME 25 · NUMBER 1 · JANUARY 1 2007

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Increased Risk of Breast Cancer Associated With *CHEK2*1100delC*

Maren Weischer, Stig Egil Bojesen, Anne Tybjærg-Hansen, Christen Kirk Axelsson, and Børge Grønne Nordestgaard

From the Department of Clinical Biochemistry, Herlev University Hospital; Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital; The Copenhagen City Heart Study, Bispebjerg University Hospital; and Department of Breast Surgery, Herlev University Hospital, University of Copenhagen, Copenhagen, Denmark.

Submitted December 27, 2005; accepted June 6, 2006; published online ahead of print at www.jco.org on July 31, 2006.

Supported by the Michaelsen Foundation, the Danish Heart Foundation, Chief Physician Johan Boserup and Lise Boserups Fund, the Danish Medical Research Council, the Research Fund at Rigshospitalet, Copenhagen University Hospital, and Copenhagen County.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Børge G. Nordestgaard, Professor and Chief Physician, Department of Clinical Biochemistry, Herlev University Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark; e-mail: brno@herlevhosp.kbhamt.dk.

0732-183X/07/2501-57/$20.00

DOI: 10.1200/JCO.2005.05.5160

ABSTRACT

Purpose
*CHEK2*1100delC* heterozygosity has been associated with increased risk of breast, prostate, and colorectal cancer in case-control studies. We tested the hypothesis that *CHEK2*1100delC* heterozygosity in the general population increases the risk of cancer in general, and breast, prostate, and colorectal cancer in particular.

Patients and Methods
We performed a prospective study of 9,231 individuals from the Danish general population, who were observed for 34 years, and we performed a case-control study including 1,101 cases of breast cancer and 4,665 controls.

Results
Of the general population, 0.5% were heterozygotes and 99.5% were noncarriers. In the prospective study, multifactorially adjusted hazard ratios by *CHEK2*1100delC* heterozygosity versus noncarriers were 1.2 (95% CI, 0.7 to 2.1) for all cancers, 3.2 (95% CI, 1.0 to 9.9) for breast cancer, 2.3 (95% CI, 0.6 to 9.5) for prostate cancer, and 1.6 (95% CI, 0.4 to 6.5) for colorectal cancer. In the case-control study, age-matched odds ratio for breast cancer by *CHEK2*1100delC* heterozygosity versus noncarriers was 2.6 (95% CI, 1.3 to 5.4). The absolute 10-year risk of breast cancer in *CHEK2*1100delC* heterozygotes amounted to 24% in women older than 60 years undergoing hormone replacement therapy, with a body mass index of 25 kg/m^2 or higher.

Conclusion
*CHEK2*1100delC* heterozygosity is associated with a three-fold risk of breast cancer in women in the general population.

J Clin Oncol 25:57-63. © 2007 by American Society of Clinical Oncology

INTRODUCTION

In most countries, women with breast cancer and a familial predisposition to this disease are offered screening for mutations in all of the *BRCA1* and *BRCA2* genes. However, in fewer than a quarter of the women screened, a causative mutation is found in either of these two genes. Besides mutations in the *BRCA1* and *BRCA2* genes, the common *CHEK2*1100delC* mutation is the most likely candidate to explain genetic risk of breast cancer.[1-9]

CHEK2 (OMIM 604373) is a key checkpoint kinase that acts as a tumor suppressor in response to DNA double-strand breakage.[1] DNA damage results in activation of cell-cycle checkpoints that block cell proliferation and initiate DNA repair.[1] Impaired function of such checkpoints can lead to genomic instability and susceptibility to cancer. Through its ability to phosphorylate *p53*, *Cdc25c*, and *BRCA1*, CHEK2 leads to cell cycle arrest or apoptosis. The first evidence suggesting the implication of CHEK2 in cancer development was the germline mutation *CHEK2*1100delC* found in several Li-Fraumeni and Li-Fraumeni–like families.[2,3]

The *CHEK2*1100delC* variant is caused by a deletion of a single cytosine at position 1100, resulting in the introduction of a stop codon after aminoacid 380,[2] and in complete loss of CHEK2 kinase activity. Apart from the association with the Li-Fraumeni syndromes, *CHEK2*1100delC* heterozygosity has also been associated with breast,[4-9] prostate,[10,11] and colorectal cancer[7,12]; *CHEK2*1100delC* heterozygosity are found in 1.1% to 1.4% of white people in Northern Europe.[6,9] These studies[4-12] were all case-control studies and the effect of *CHEK2*1100delC* heterozygosity in the general population has never previously been examined.

We tested the hypothesis that *CHEK2*1100delC* heterozygosity in the general population increases

the risk of cancer in general, and breast, prostate, and colorectal cancer in particular. For this purpose, we genotyped 9,231 individuals from the Danish general population who were observed for 34 years, and the risk of 24 other cancer subtypes were examined in exploratory analyses. Furthermore, we also genotyped 1,101 consecutively collected female breast cancer patients from a large surgical department and compared them with 4,665 female controls from the general population.

PATIENTS AND METHODS

Study Populations

For the prospective study, we examined 9,231 individuals (randomly selected after age and sex stratification) from the Danish general population who participated in the 1991 to 1994 Copenhagen City Heart Study[13-15] (56% of those invited participated in our study). Participants were interviewed and examined in the years 1976 to 1978, 1981 to 1983 and 1991 to 1994, and participants' medical history, family history of disease, alcohol consumption, smoking habits, and reproductive history (women only) were noted. Height and weight were measured at every examination. Blood samples for DNA extraction were drawn at the 1991 to 1994 examination. More than 99% of the participants were white and of Danish descent. Diagnoses of invasive cancer (diagnoses were made using WHO International Classification of Diseases, seventh edition; ICD-7[14]) for the whole cohort from 1947 until December 31, 2002, were obtained from the Danish Cancer Registry,[16,17] which identifies 98% of all cancers in Denmark.[18] Cancer diagnoses were divided in 27 subgroups according to WHO criteria.[19] ICD-7 codes 170.0 to 170.5, 470.0 to 470.1, and 970.0 to 970.1 were classified as breast cancer; 177.0, 477.0, and 977.0 were classified as prostate cancer; 91.0, 153.0 to 153.4, 154. 0, 154.9, 253.0 to 253.4, 453.0 to 453.5, 453.8, 454.0, 554.0, 953.0 to 953.4, and 954.0 were classified as colorectal cancer; 93.1, 93.3 to 93.7, 193.1 to 193.2, 195.4, 293.0 to 293.2, 393.1, 493.0, 493.2 to 493.3, 493.5 to 493.7, and 993.0 to 993.1 were classified as brain/nerve tissue cancers; 204.0 to 204.4, 214.0 to 214.1, 404.0, 503.0, 504.4, 904.4, and 914.1 were classified as leukemia; and 180.0, 180.3, 980.0, and 980.3 were classified as kidney cancer. Follow-up time for each participant began at the establishment of the Danish Civil Register System April 1, 1968, or the participant's 20th birthday, whichever came later, and ended at death, event, emigration, or on December 31st, 2002, whichever came earlier. Participants with disease before or after follow-up were excluded. This included 141 events of any cancer, 17 of breast cancer, six of prostate cancer, 12 of colorectal cancer, three of brain/nerve tissue cancers, two of leukemia, and none of kidney cancer. The maximum and median follow-up periods were 34.7 and 33.8 years, respectively. Follow-up was 100% complete. Death due to intercurrent disease was treated as censoring.

The case-control study included 1,101 women with invasive breast cancer consecutively recruited at Herlev University Hospital (Herlev, Denmark) between February 2001 and August 2004 (98% of those invited participated in our study). Participants gave blood and filled out questionnaires regarding medical history, family history of breast cancer, alcohol consumption, use of oral contraceptives, use of hormonal replacement therapy, reproductive history, height, and weight. More than 97% of the participants were white and of Danish descent. Information on tumor characteristics and dissemination was obtained from The Danish Breast Cancer Group.[20] Controls were 4,665 women from the general population (The Copenhagen City Heart Study) within the same age range as the patients, who had no history of breast cancer before the end of 2002.

Ethics

All participants gave written informed consent. Herlev University Hospital and Danish ethical committees approved the studies (No. 100.2039/91, KA02152).

Genotyping

Leukocyte DNA was used to amplify a 251 base pair (bp) long fragment flanking the *CHEK2*-position 1100 bp in exon 10 by polymerase chain reaction (PCR) using two primers (sense, carboxyfluorescein-labeled, 5′-TAA TTT AAG CAA AAT TAA ATG TCC-3′; antisense 5′-GTT CCA CAT AAG GTT CTC AT-3′). Due to *CHEK2*-pseudogenes on chromosomes 15 and 16, we paid careful attention to avoid pseudogene sequences in the 3′ ends of the primers. Fragment length of the PCR product was determined by the Megabase 500 system (GE Healthcare, Hilleroed, Denmark), exploiting the length difference of 1 bp between the normal and variant allele. All heterozygotes were sequenced to confirm the genotype, on an independent PCR reaction. All participants were genotyped using identical procedures run in the same lab. Each run included positive and negative controls.

Statistical Analyses

We used the statistical software STATA (STATA/SE for Windows, version 8.2; Stata Corp, College Station, TX). Two-sided $P < .05$ was significant. We used the Mann-Whitney U test or Pearson's χ^2 test.

In the prospective study, we used Cox regression with delayed entry and used age as the underlying time variable; we thus automatically adjusted for age. Multifactorially adjusted models also included time-dependent covariates from the 1976 to 1978, 1981 to 1983 and 1991 to 1994 examinations. Multifactorial adjustment for breast cancer included age, body mass index (< 25 *v* ≥ 25 kg/m^2), alcohol consumption (0 g/wk *v* > 0 g/wk), nulliparity (yes *v* no), current use of oral contraceptives (yes *v* no), menopausal status (pre- *v* postmenopausal), and current use of hormone replacement therapy (yes *v* no). Multifactorial adjustment for colorectal cancer included age, sex, body mass index (< 25 *v* ≥ 25 kg/m^2), smoking (current smoker *v* nonsmoker), alcohol consumption (0 g/wk *v* > 0 g/wk), and smoking history (ever smoker *v* never smoker). Multifactorial adjustment for brain/nerve tissue cancers, leukemia, and kidney cancer included age and sex, and adjustments for prostate cancer only included age. The proportional hazards assumption based on Schoenfeld residuals was appropriate for all comparisons. A two-factor interaction term tested for interaction in the Cox regression.

The case-control study was matched with a 1-year age strata to perform conditional logistic regression. This resulted in 64 strata with a mean of 4.2 controls per patient. A two-factor interaction term tested for interaction in the logistic regression model.

Population attributable risk was estimated as $[f(HR-1)]/[1+f(HR-1)]$, where f is the frequency of *CHEK2*1100delC* in the population and HR is the hazard ratio for breast cancer.[21]

Absolute risks for breast cancer by *CHEK2*1100delC* heterozygosity were estimated by using the regression coefficients from a Poisson regression model with the following covariates: body mass index in two groups (< 25 kg/m^2 *v* ≥ 25 kg/m^2), age in three groups (< 40 years, 40 to 60 years, and > 60 years), and use of hormone replacement therapy (no *v* yes) at the date of blood sampling. Absolute risks are presented as estimated incidence rates (events/10 years) in percentages.

Role of the Funding Organizations

The funding organizations had no role in the design or conduct of the study, or in the collection, management, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

RESULTS

Prospective Study

Of the general population, 0.5% were *CHEK2*1100delC* heterozygotes and 99.5% were noncarriers. No homozygotes were identified. This distribution was in Hardy-Weinberg equilibrium (χ^2 test, $P = .81$). Characteristics of the participants are listed in Table 1. There were no significant differences between heterozygotes and noncarriers for any of these established risk factors for cancer in either study (data not shown).

Hypotheses Testing

During the 34 years of follow-up, we detected 1,914 participants with a first incidence of cancer (Table 1). Cancer incidences for

Table 1. Participant Characteristics

	Prospective Study		Case-Control Study	
	Cancer (n = 1,914)	Without Cancer (n = 7176)	Breast Cancer (n = 1,101)	Controls (n = 4,665)
Women, %	54	55	100	100
Age, years				
Median	53*	45	62	62
Range	32-70	23-67	36-85	31-83
Smoker, %	65*	59	—	—
Body mass index, kg/m^2				
Median	25*	24	24*	25
Range	19-34	19-34	18-35	19-36
Alcohol consumption, g/wk				
Median	0	0	48	36
Range	0-588	0-511	0-252	0-300
Nulliparous (women only), %	21†	25	13*	20
Oral contraceptive use (women only), %	5*	11	3	3
Postmenopausal (women only), %	59*	35	76*	73
Hormonal replacement therapy (women only), %	24*	13	31*	16

NOTE. Values represent numbers of patients unless otherwise indicated. Medians are measured from the 2.5-97.5 percentiles. In the prospective study, values are at entry, whereas in the case-control study, values are at recruitment for cases and at the 1991-1994 examination for controls.
*$P < .001$ versus participants without cancer/controls using Mann-Whitney U test or χ^2 test.
†$P < .05$.

noncarriers and heterozygotes, for both sexes combined, were 70 and 114 per 10,000 person-years, respectively. Equivalent values were 68 and 66 in women, respectively, and 71 and 180 in men, respectively. The age-adjusted hazard ratios for heterozygotes versus noncarriers were 1.4 (95% CI, 0.9 to 2.4) for men and women combined, 1.0 (95% CI, 0.4 to 2.3) for women alone, and 1.8 (95% CI, 1.0 to 3.4) for men alone. After multifactorial adjustment for the covariates that have been listed in Table 1, the equivalent hazard ratios were 1.2 (95% CI, 0.7 to 2.1) for men and women combined, 1.1 (95% CI, 0.4 to 2.6) for women alone, and 1.5 (95% CI, 0.7 to 2.9) for men alone. Genotype and sex did not interact with the risk of all cancer ($P = .20$).

Among women, incidences of breast cancer in noncarriers and *CHEK2*1100delC* heterozygotes were 17 and 39 per 10,000 person-years, respectively (Table 2). The age-adjusted hazard ratio of breast cancer for heterozygotes versus noncarriers was 2.5 (95% CI, 0.8 to 7.7), which increased to 3.2 (95% CI, 1.0 to 9.9) after multifactorial

Table 2. Incidence and Risk of Site-Specific Cancers According to *CHEK2*1100delC* Genotype by Cox Regression

	No. of Participants	No. of Cancers	Incidence per 10,000 Person-Years	95% CI	Age Adjustment		Multifactorial Adjustment	
					Hazard Ratio	95% CI	Hazard Ratio	95% CI
Breast cancer, women only								
Noncarriers	5,066	273	17	15 to 19	1.0		1.0	
Heterozygotes	25	3	39	13 to 121	2.5	0.8 to 7.7	3.2	1.0 to 9.9
Prostate cancer, men only								
Noncarriers	4,094	114	9	7 to 11	1.0		1.0	
Heterozygotes	21	2	31	8 to 123	2.3	0.6 to 9.5	2.3	0.6 to 9.5
Colorectal cancer, women and men								
Noncarriers	9,171	208	7	6 to 8	1.0		1.0	
Heterozygotes	46	2	14	3 to 56	1.7	0.4 to 6.7	1.6	0.4 to 6.5
Brain/nervous tissue cancers, women and men								
Noncarriers	9,180	36	1	1 to 2	1.0		1.0	
Heterozygotes	46	2	14	3 to 55	10.1	2.4 to 41.9	9.9	2.4 to 41.2
Leukemia, women and men								
Noncarriers	9,181	48	2	1 to 2	1.0		1.0	
Heterozygotes	46	2	14	3 to 55	6.7	1.6 to 27.7	6.3	1.5 to 26.1
Kidney cancer, women and men								
Noncarriers	9,183	30	1	1 to 1	1.0		1.0	
Heterozygotes	46	3	14	3 to 56	10.8	2.6 to 45.7	9.8	2.3 to 41.2

NOTE. Numbers vary slightly due to difference in the number of participants with disease before and after follow-up.

adjustment. Among men, incidences of prostate cancer in noncarriers versus heterozygotes were 9 and 31 per 10,000 person-years, respectively. The age-adjusted hazard ratio of prostate cancer for heterozygotes versus noncarriers was 2.3 (95% CI, 0.6 to 9.5). In women and men combined, incidences of colorectal cancer in noncarriers versus heterozygotes were 7 and 14 per 10,000 person-years, respectively. Age-adjusted and multifactorially adjusted hazard ratios of colorectal cancer in heterozygotes versus noncarriers were 1.7 (95% CI, 0.4 to 6.7) and 1.6 (95% CI, 0.4 to 6.5).

Exploratory Analyses

Incidences of brain/nerve tissue cancer in noncarriers versus heterozygotes were 1 and 14 per 10,000 person-years, respectively (Table 2). Age-adjusted and multifactorially adjusted hazard ratios for brain or nerve tissue cancers in heterozygotes versus noncarriers were 10.1 (95% CI, 2.4 to 41.9) and 9.9 (95% CI, 2.4 to 41.2). Incidences of leukemia in noncarriers versus heterozygotes were 2 and 14 per 10,000 person-years, respectively. Age-adjusted and multifactorially adjusted hazard ratios for leukemia in heterozygotes versus noncarriers were 6.7 (95% CI, 1.6 to 27.7) and 6.3 (95% CI, 1.5 to 26.1). Incidences of kidney cancer in noncarriers versus heterozygotes were 1 and 14 per 10,000 person-years, respectively. Age-adjusted and multifactorially adjusted hazard ratios of kidney cancer in heterozygotes versus noncarriers were 10.8 (95% CI, 2.6 to 45.7) and 9.8 (95% CI, 2.3 to 41.2). *CHEK2*1100delC* heterozygosity was not associated with increased risk of any of the remaining 21 site-specific cancers.

Case-Control Study

The overall odds ratio for invasive breast cancer in heterozygotes versus noncarriers was 2.6 (95% CI, 1.3 to 5.4; Fig 1). After stratification, odds ratios were 2.8 (95% CI, 1.0 to 7.9) in women older than 60 years, 3.5 (95% CI, 1.4 to 9.0) in women with body mass indexes of 25 kg/m^2 or higher, 3.0 (95% CI, 1.4 to 6.5) in women with any weekly alcohol intake, 3.2 (95% CI, 1.4 to 7.1) in postmenopausal women, and 6.7 (95% CI, 1.4 to 32.7) in women currently using hormone replacement therapy. In the other contexts examined, odds ratios were not significant. Genotype did not, however, interact significantly with any of the contexts examined (Fig 1).

After stratification for histologic subtypes of breast cancer, the odds ratio was 2.3 (95% CI, 1.0 to 5.5) for ductal tumor; for other histologic subtypes, the odds ratios were nominally higher, but not statistically significant (Table 3). After stratification for tumor characteristics and dissemination at diagnosis, odds ratios for breast cancer in heterozygotes versus noncarriers were 5.3 (95% CI, 1.5 to 19.0) in those patients with tumor size ≤ 10 mm, 4.3 (95% CI, 1.2 to 15.3) and 3.0 (95% CI, 1.2 to 7.7) in those patients with grade 1 and unknown grades of malignancy in ductal carcinoma, 3.4 (95% CI, 1.6 to 7.5) in those patients with hormone-receptor–negative tumors, 5.3 (95% CI, 1.9 to 14.7) in those patients with tumor-positive lymph nodes without breakthrough of capsule, and 2.6 (95% CI, 1.2 to 5.8) in those patients with absent distant metastases (Table 4). In the other subgroups examined, the odds ratios were all higher than 1.0, but were not statistically significant.

Population-Attributable Risk of Breast Cancer

Based on a frequency of 0.5% for *CHEK2*1100delC* in the general population and a hazard ratio of 3.2 for breast cancer in female heterozygotes versus noncarriers, the population-attributable risk of breast cancer in women for *CHEK2*1100delC* was 1.1%.

Absolute Risk of Breast Cancer

The lowest absolute 10-year risk for breast cancer was 2% in female *CHEK2*1100delC* heterozygotes who were younger than 40 years old, with a body mass index lower than 25 kg/m^2 (Fig 2). Absolute risk increased with increasing age, use of hormone replacement therapy, and a body mass index at or higher than 25 kg/m^2. The highest absolute 10-year risk for breast cancer in *CHEK2*1100delC* heterozygotes was 24% in hormone replacement therapy users older than 60 years old with a body mass index at or higher than 25 kg/ m^2.

DISCUSSION

CHEK2 is part of a phylogenetically conserved pathway that is activated in response to DNA damage.[1] This, together with the fact that a recent case-control study found an association between mutations in *CHEK2* (IVS2+1G→A and 1100delC) and thyroid, breast, and prostate cancer, as well as between another variant (I157T) and breast, colon, kidney, prostate, and thyroid cancer,[22] support the hypothesis that *CHEK2* may be a general cancer susceptibility gene. However, our

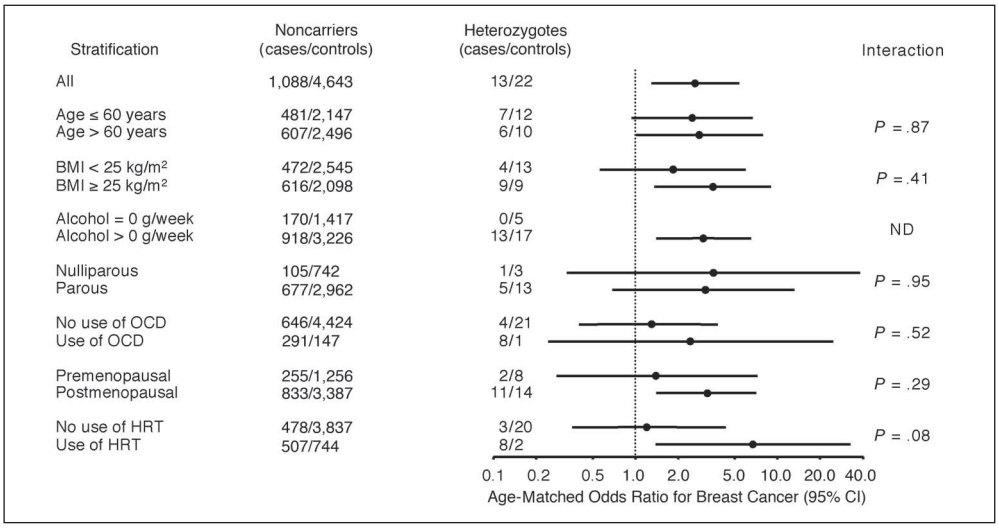

Fig 1. *CHEK2*1100delC* associated with breast cancer in the case-control study. Stratification by age, body mass index (BMI), alcohol consumption, parity, use of oral contraceptives (OCD), menopausal status, and use of hormonal replacement therapy (HRT). Numbers vary slightly due to incomplete information for some of the covariates used for stratification. ND, not determined.

Table 3. Risk of Invasive Breast Cancer by *CHEK2*1100delC* Genotype in Case-Control Study

	No. of Noncarriers*	No. of Heterozygotes†	Age-Matched Odds Ratio	95% CI
All	1,088	13	2.6	1.3 to 5.4
Histologic subtype				
Ductal	643	7	2.3	1.0 to 5.5
Lobular	104	2	4.0	0.9 to 17.9
Others	48	1	6.2	0.7 to 56.9
Unknown	293	3	2.4	0.7 to 8.2

*No. of controls = 4,663; all controls were included in each calculation.
†No. of controls = 22; all controls were included in each calculation.

study in the Danish general population following 9,231 individuals for 34 years, during which 1,914 incident cancers developed, was not able to support this hypothesis.

The contribution of hereditary factors to the causation of breast cancer is 27% (95% CI, 4% to 41%).[23] *BRCA1* and *BRCA2* genes account for only 3% to 8% of all breast cancers in women.[24] In our prospective study, we found a carrier frequency of *CHEK2*1100delC* of 0.5% in the general population, similar to that observed in controls from Germany.[25] The 3.2-fold risk of breast cancer in *CHEK2*1100delC* heterozygotes found in the general population is similar to the 2.6-fold risk observed in our large case-control study, and to the 2.3-fold risk reported in a large multicenter case-control study by the *CHEK2* Breast Cancer Consortium.[4] The 3.2-fold risk and a frequency of 0.5% results in a population-attributable risk of breast cancer in women of 1.1% for *CHEK2*1100delC*, or slightly less than the total of 3% to 8%, accounted for by all mutations in *BRCA1* and *BRCA2*.[24] In populations with a *CHEK2*1100delC* heterozygosity frequency of 1.4%, like in Finland,[9] a 3.2-fold risk of breast cancer would imply a 3.0% population-attributable risk of breast cancer in women.

Our observation of positive odds ratios for breast cancer by *CHEK2*1100delC* heterozygosity in all strata, although some were not statistically significant, together with no evidence of statistical interaction between genotypes and context on breast cancer risk, support the

Table 4. Risk of Breast Cancer by *CHEK2*1100delC* Genotype Stratified by Tumor Characteristics and Dissemination at Diagnosis in Case-Control Study

	No of Noncarriers*	No of Heterozygotes†	Age-Matched Odds Ratio	95% CI
Tumor size, mm				
≤ 10	141	3	5.3	1.5 to 19.0
11-20	314	3	1.9	0.5 to 6.4
21-30	222	3	2.9	0.8 to 10.0
31-50	89	1	2.1	0.3 to 17.0
≥ 50	32	0	—	
Unknown	290	3	2.4	0.7 to 8.2
Grade of malignancy (only ductal)				
Grade I	163	3	4.3	1.2 to 15.3
Grade II	310	4	2.8	0.9 to 8.3
Grade III	152	0	—	
Unknown	18	6	3.0	1.2 to 7.7
Estrogen receptor status				
Positive	131	0	—	
Negative	639	10	3.4	1.6 to 7.5
Unknown	318	3	2.1	0.6 to 7.3
Lymph node involvement				
No tumor nodes	392	3	1.7	0.5 to 6.0
Tumor positives nodes, without breakthrough of capsule	206	5	5.3	1.9 to 14.7
Tumor positives nodes, with breakthrough of capsule	188	2	2.0	0.4 to 8.6
Unknown	302	3	2.3	0.7 to 7.8
Distant metastases				
Absent	752	9	2.6	1.2 to 5.8
Present	5	0	—	
Unknown	331	4	2.6	0.9 to 7.7

*No. of controls = 4,633; all controls were included in each calculation.
†No. of controls = 22; all controls were included in each calculation.

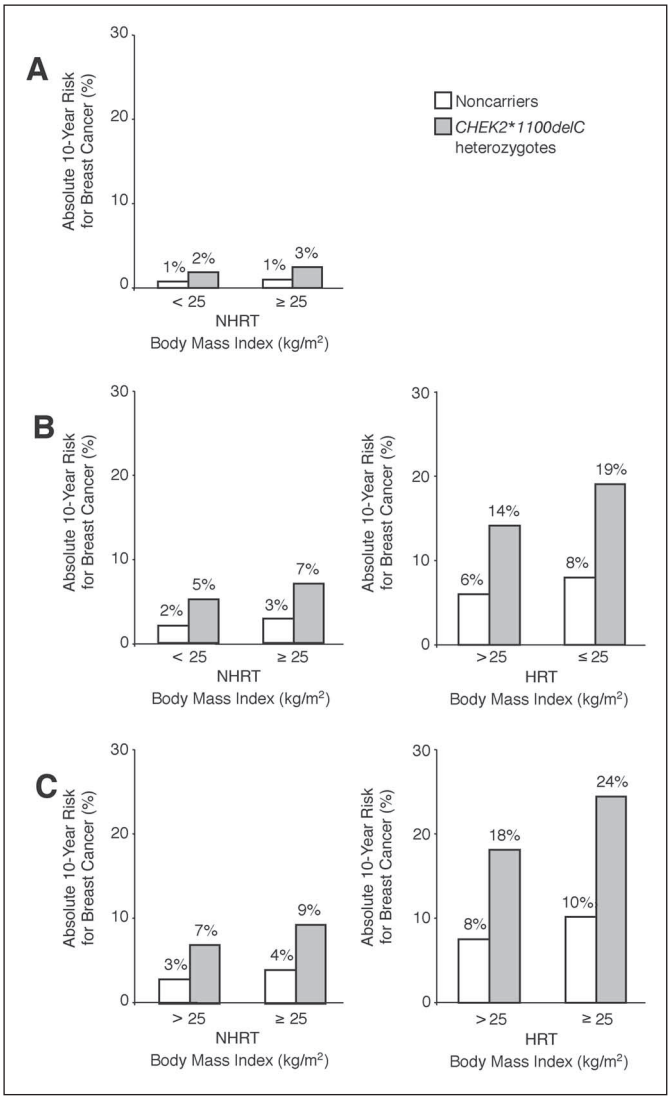

Fig 2. Absolute 10-year risk for invasive breast cancer in women according to age, use of hormone replacement therapy (HRT), body mass index, and *CHEK2*1100delC* genotype. (A) Women younger than 40 years; (B) women 40-60 years old; (C) women older than 60 years. NHRT, no hormone replacement therapy.

interpretation that women heterozygous for *CHEK2*1100delC* have an increased risk of breast cancer regardless of age, body mass index, alcohol consumption, parity, use of oral contraceptives, menopausal status, and use of hormone replacement therapy.

*CHEK2*1100delC* heterozygosity has been associated with an odds ratio of 1.7 to 2.1 in unselected Polish prostate cancer patients, increasing to 4.9 to 8.2 in Polish and Finnish heterozygotes with a positive family history of this disease.[10,11,22] Our findings of a hazard ratio of 2.3 (95% CI, 0.6 to 9.5) for prostate cancer by *CHEK2*1100delC* heterozygosity, although not statistically significant, is in line with these results.

Conflicting reports regarding the association between *CHEK2*1100delC* heterozygosity and colorectal cancer have been published previously; *CHEK2*1100delC* was first associated with colorectal cancer in Dutch families with hereditary breast and colorectal cancer,[7] whereas case-control studies in the Finland, Poland, and the United Kingdom reported no such association.[12,22,26] Although *CHEK2*1100delC* heterozygosity conferred a hazard ratio of 1.6 (95% CI, 0.4 to 6.5) for colorectal cancer in our study, this was not statistically significant.

To the best of our knowledge, we are the first to report an association between *CHEK2*1100delC* heterozygosity and brain and/or nerve tissue cancers. Previously, only one study examined the association between *CHEK2*1100delC* heterozygosity and leukemia; Collado et al examined 107 Spanish leukemia patients and found no *CHEK2*1100delC* heterozygotes[27]; however, *CHEK2*1100delC* is nearly absent from the Spanish population, and this may explain their finding.[27,28] Therefore, other studies are needed to confirm (or rebut) our findings of an association between *CHEK2*1100delC* and brain/nerve tissue cancers and leukemia.

The association between *CHEK2*1100delC* and kidney cancer has been examined by Cybulski et al,[22] who observed an insignificant odds ratio of 2.7 ($P = .50$). This is well below the hazard ratio of 9.8 that was observed in our prospective study; interestingly, however, that same article reported an odds ratio of 2.1 ($P < .001$) for kidney cancer by the *CHEK2 I157T* variant versus noncarriers.[22]

There are some limitations to our study, First, the number of *CHEK2*1100delC* heterozygotes is limited. Second, only participants attending the 1991 to 1994 examination of the Copenhagen City Heart Study were genotyped. A selection bias might have occurred if death or morbidity prevented certain participants from attending the 1991 to 1994 examination. However, two observations make substantial selection bias against any genotype less likely. (1) In the general population, the frequency of noncarriers and heterozygotes do not increase or decrease as a function of age. (2) The distribution between genotypes was in Hardy-Weinberg equilibrium.

Third, misclassification of disease may have occurred. However, this is not very likely because we have 100% follow-up of participants, and because all hospital admissions and deaths are registered in the country. Furthermore, the Danish National Cancer Registry identifies 98% of all cancers in Denmark.[18]

A likely limitation of case-control studies is the selection of controls; however, we chose breast cancer–free female participants from the Danish general population as controls. Furthermore, cases and controls came from the same geographic area and were matched for age.

Our participants could be affected by selection bias. However, this is less likely to have occurred because, (1) participants were consecutively collected, and (2) blood samples were drawn within 30 days of breast cancer diagnosis in more than 80% of the cases, practically excluding that *CHEK2*1100delC* heterozygotes died selectively compared with noncarriers.

Women with *CHEK2*1100delC* may benefit from preventive examinations for breast cancer, preferably excluding ionizing radiation.[29] In contrast with screening all *BRCA1* and *BRCA2* genes, *CHEK2*1100delC* testing is a single genotyping test that costs less than US$10, which detects 100% of carriers. Cost-effectiveness analyses should evaluate whether *CHEK2*1100delC* genotyping followed by preventive examinations should be offered to only women with high risk of familial breast cancer or sporadic breast cancer, and/or who have other risk factors (Fig 2), or if it should be offered to most women.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Maren Weischer, Stig E. Bojesen, Anne Tybjærg-Hansen, Børge G. Nordestgaard
Financial support: Anne Tybjærg-Hansen, Børge G. Nordestgaard
Administrative support: Børge G. Nordestgaard
Provision of study materials or patients: Stig E. Bojesen, Anne Tybjærg-Hansen, Christen K. Axelsson, Børge G. Nordestgaard
Collection and assembly of data: Maren Weischer, Stig E. Bojesen, Anne Tybjærg-Hansen, Christen K. Axelsson, Børge G. Nordestgaard
Data analysis and interpretation: Maren Weischer, Stig E. Bojesen, Anne Tybjærg-Hansen, Børge G. Nordestgaard
Manuscript writing: Maren Weischer, Stig E. Bojesen
Final approval of manuscript: Stig E. Bojesen, Anne Tybjærg-Hansen, Christen K. Axelsson, Børge G. Nordestgaard

REFERENCES

1. Bartek J, Lukas J: Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 3:421-429, 2003
2. Bell DW, Varley JM, Szydlo TE, et al: Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 286:2528-2531, 1999
3. Vahteristo P, Tamminen A, Karvinen P, et al: p53, CHK2, and CHK1 genes in Finnish families with Li-Fraumeni syndrome: Further evidence of CHK2 in inherited cancer predisposition. Cancer Res 61: 5718-5722, 2001
4. CHEK2 Breast Cancer Case-Control Consortium: CHEK2*1100delC and susceptibility to breast cancer: A collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 74:1175-1182, 2004
5. Kilpivaara O, Bartkova J, Eerola H, et al: Correlation of CHEK2 protein expression and c1100delC mutation status with tumor characteristics among unselected breast cancer patients. Int J Cancer 113:575-580, 2005
6. Meijers-Heijboer H, van den OA, Klijn J, et al: Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 31:55-59, 2002
7. Meijers-Heijboer H, Wijnen J, Vasen H, et al: The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. Am J Hum Genet 72:1308-1314, 2003
8. Oldenburg RA, Kroeze-Jansema K, Kraan J, et al: The CHEK2*1100delC variant acts as a breast cancer risk modifier in non-BRCA1/BRCA2 multiple-case families. Cancer Res 63:8153-8157, 2003
9. Vahteristo P, Bartkova J, Eerola H, et al: A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 71:432-438, 2002
10. Cybulski C, Huzarski T, Gorski B, et al: A novel founder CHEK2 mutation is associated with increased prostate cancer risk. Cancer Res 64:2677-2679, 2004
11. Seppala EH, Ikonen T, Mononen N, et al: CHEK2 variants associate with hereditary prostate cancer. Br J Cancer 89:1966-1970, 2003
12. Kilpivaara O, Laiho P, Aaltonen LA, et al: CHEK2 1100delC and colorectal cancer [letter]. J Med Genet 40:e110, 2003 (suppl 10)
13. Appleyard M, Hansen AT, Jensen G, et al: The Copenhagen City Heart Study: Østerbroundersøgelsen—A book of tables with data from the first examination (1976-78) and a five year follow-up (1981-1983). Scand J Soc Med Suppl 41:1-160, 1989
14. Bojesen SE, Tybjærg-Hansen A, Nordestgaard BG: Integrin beta3 Leu33Pro homozygosity and risk of cancer. J Natl Cancer Inst 95:1150-1157, 2003
15. Schnohr P, Jensen G, Lange P, et al: The Copenhagen City Heart Study: Østerbroundersøgelsen—Tables with data from the third examination 1991-1994. Eur Heart J 3:H1-H83, 2001
16. Storm HH: The Danish Cancer Registry, a self-reporting national cancer registration system with elements of active data collection. IARC Sci Publ 95:220-236, 1991
17. Storm HH, Michelsen EV, Clemmensen IH, et al: The Danish Cancer Registry: History, content, quality and use. Dan Med Bull 44:535-539, 1997
18. Storm HH: Completeness of cancer registration in Denmark 1943-1966 and efficacy of record linkage procedures. Int J Epidemiol 17:44-49, 1988
19. Bray F, Sankila R, Ferlay J, et al: Estimates of cancer incidence and mortality in Europe in 1995. Eur J Cancer 38:99-166, 2002
20. Danish Breast Cancer Cooperative Group: Structure and organisation of DBCG, subchapters 3.1-3.3. http://www.dbcg.dk
21. Khoury MJ, Beaty TH, Cohen BH: Fundamentals of Genetic Epidemiology. New York, NY, Oxford University Press, 1993, pp 77-79
22. Cybulski C, Gorski B, Huzarski T, et al: CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet 75:1131-1135, 2004
23. Lichtenstein P, Holm NV, Verkasalo PK, et al: Environmental and heritable factors in the causation of cancer: Analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343:78-85, 2000
24. Brody LC, Biesecker BB: Breast cancer susceptibility genes: BRCA1 and BRCA2. Medicine (Baltimore) 77:208-226, 1998
25. Dufault MR, Betz B, Wappenschmidt B, et al: Limited relevance of the CHEK2 gene in hereditary breast cancer. Int J Cancer 110:320-325, 2004
26. Lipton L, Fleischmann C, Sieber OM, et al: Contribution of the CHEK2 1100delC variant to risk of multiple colorectal adenoma and carcinoma. Cancer Lett 200:149-152, 2003
27. Collado M, Landt O, Barragan E, et al: Locked nucleic acid-enhanced detection of 1100delC*CHEK2 germ-line mutation in Spanish patients with hematologic malignancies. Clin Chem 50:2201-2204, 2004
28. Osorio A, Rodriguez-Lopez R, Diez O, et al: The breast cancer low-penetrance allele 1100delC in the CHEK2 gene is not present in Spanish familial breast cancer population. Int J Cancer 108:54-56, 2004
29. Bernstein JL, Teraoka SN, John EM, et al: The CHEK2*1100delC allelic variant and risk of breast cancer: Screening results from the Breast Cancer Family Registry. Cancer Epidemiol Biomarkers Prev 15:348-352, 2006

Acknowledgment

Nina Dahl Kjersgaard provided technical assistance.